

-continued

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Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile
      260                      265                      270

Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn
      275                      280                      285

Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro
      290                      295                      300

Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp
      305                      310                      315                      320

Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met
      325                      330                      335

Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala
      340                      345                      350

Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys
      355                      360                      365

Asp Ala Gln Thr Asn
      370

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<210> SEQ ID NO 72
<211> LENGTH: 17
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: RBS

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<400> SEQUENCE: 72

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aaaacaaguu auccaug

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17

1-24. (canceled)

25. A variant protease, wherein said variant protease cleaves a protease cleavage site (PCS) having the amino acid sequence of SEQ ID NO: 41 (Mut1 bdSUMO) and fused to the N-terminus of MBP having the amino acid sequence of SEQ ID NO: 71 more efficiently after the C-terminal Gly-Gly motif than a protease cleavage site having the amino acid sequence of SEQ ID NO: 4 (scSUMO) fused to the N-terminus of SEQ ID NO: 71 or a protease cleavage site having the amino acid sequence of SEQ ID NO: 3 (hs-SUMO2) fused to the N-terminus of SEQ ID NO: 71, when tested at the same concentration under standard conditions of 1 hour incubation at 21° C., an initial concentration of PCS-MBP fusions of 100 µM in a buffer consisting of 45 mM Tris/HCl pH 7.5, 250 mM NaCl, 2 mM MgCl₂, 250 mM sucrose, 10 mM DTT.

26. The variant protease of claim **25**, wherein said variant protease cleaves an at least a 500-fold molar excess of the Mut1 bdSUMO-MBP fusion at the above standard conditions.

27. The variant protease of claim **25**, wherein said variant protease has at least 80% sequence identity over the full-length of SEQ ID NO: 6 (bdSEN1), wherein said variant protease, when aligned to the full-length sequence of SEQ ID NO: 6, comprises a substitution at the position corresponding to N280 of the aligned SEQ ID NO: 6, wherein the amino acid at said position is substituted by an amino acid selected from the group consisting of S, H, Q, A, G, and C.

28. The variant protease of claim **27**, wherein the amino acid is selected from the group consisting of S, H, Q, and A.

29. The variant protease of claim **27**, wherein said variant protease, when aligned to the full-length sequence of SEQ ID NO: 6, further comprises a substitution at the position corresponding to R356 of the aligned SEQ ID NO: 6, wherein the amino acid at said position is substituted by another amino acid selected from the group consisting of E, S, V, Y, and L.

30. The variant protease of claim **29**, wherein the substitution is selected from the group consisting of E, S, and V.

31. The variant protease of claim **29**, wherein said variant protease, when aligned to the full-length sequence of SEQ ID NO: 6, further comprises a substitution at the position corresponding to R269 of the aligned SEQ ID NO: 6, wherein the amino acid at said position is substituted by another amino acid selected from the group consisting of E, S, P, K, and V.

32. The variant protease of claim **29**, wherein said variant protease, when aligned to the full-length sequence of SEQ ID NO: 6, further comprises a substitution at the position corresponding to K350 of the aligned SEQ ID NO: 6, wherein the amino acid at said position is substituted by another amino acid selected from the group consisting of M, E, V, G, T, and R.

33. The variant protease of claim **32** wherein the substitution is selected from the group consisting of M, E, V, G and T.

34. The variant protease of claim **27**, wherein said variant protease, when aligned to the full-length sequence of SEQ ID NO: 6, further comprises amino acid substitution(s) at two or three positions selected from the group of R356, R269 and K350 of the aligned SEQ ID NO: 6, wherein